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Nitrous Oxide Emissions from Corn–Soybean Systems in the Midwest

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ABSTRACT

Soil N_2O emissions from three corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] systems in central Iowa were measured from the spring of 2003 through February 2005. The three managements systems evaluated were full-width tillage (fall chisel plow, spring disk), no-till, and no-till with a rye (*Secale cereale* L. 'Rymin') winter cover crop. Four replicate plots of each treatment were established within each crop of the rotation and both crops were present in each of the two growing seasons. Nitrous oxide fluxes were measured weekly during the periods of April through October, biweekly during March and November, and monthly in December, January, and February. Two polyvinyl chloride rings (30-cm diameter) were installed in each plot (in and between plant rows) and were used to support soil chambers during the gas flux measurements. Flux measurements were performed by placing vented chambers on the rings and collecting gas samples 0, 15, 30, and 45 min following chamber deployment. Nitrous oxide fluxes were computed from the change in N_2O concentration with time, after accounting for diffusional constraints. We observed no significant tillage or cover crop effects on N_2O flux in either year. In 2003 mean N_2O fluxes were 2.7, 2.2, and 2.3 kg N_2O-N ha^{-1} yr^{-1} from the soybean plots under chisel plow, no-till, and no-till + cover crop, respectively. Emissions from the chisel plow, no-till, and no-till + cover crop plots planted to corn averaged 10.2, 7.9, and 7.6 kg N_2O-N ha^{-1} yr^{-1}, respectively. In 2004 fluxes from both crops were higher than in 2003, but fluxes did not differ among the management systems. Fluxes from the corn plots were significantly higher than from the soybean plots in both years. Comparison of our results with estimates calculated using the Intergovernmental Panel on Climate Change default emission factor of 0.0125 indicate that the estimated fluxes underestimate measured emissions by a factor of 3 at our sites.

Nitrous oxide is a major greenhouse gas that contributes approximately 6% to the total radiative forcing of the earth's atmosphere (Intergovernmental Panel on Climate Change, 2001). Anthropogenic activities are a major source of N_2O to the atmosphere, and it has been estimated that of the total global N_2O emissions in 1994 (17.6 Tg N), approximately 5.6 Tg were attributed to agricultural activities (Kroeze et al., 1999).

Over the past 25 years agricultural impacts on soil N_2O emissions have been extensively studied. It is generally recognized that management practices such as fertilizer (type, timing, application), crop, tillage, residue management, and water (precipitation, irrigation) influence N_2O emissions from agricultural soils. Breitenbeck et al. (1980) found that urea and ammonium sulfate fertilizers stimulated N_2O fluxes to a greater extent than calcium nitrate. In a subsequent study, Bremner et al. (1981) observed that injected anhydrous ammonia stimulated N_2O fluxes to an even greater extent. In a review of 104 experiments, Eichner (1990) reported that there was a trend of increasing N_2O emissions in response to increased fertilizer N additions, with anhydrous ammonium supporting the highest fluxes.

In addition to fertilizer N form, placement, and amount, other factors related to fertilizer N application have been shown to impact soil N_2O emissions. Sehy et al. (2003) observed an interaction between site-specific fertilizer additions and N_2O fluxes, with a 16% reduction in fertilizer addition to some areas of the field (125 kg N ha^{-1}) resulting in a 34% reduction in N_2O emissions in those areas (2.3 kg N_2O-N ha^{-1}). The timing of fertilizer N addition in relation to tillage and residue management also influences N_2O fluxes, as shown by Hao et al. (2001), who observed that treatments fertilized and plowed in the fall exhibited higher N_2O fluxes than either fall fertilized no-till treatments or spring fertilized, spring chisel plow treatments. These differences were attributed to the temporal effects of fertilizer, and residue incorporation on N mineralization and denitrification.

Observations from previous studies illustrate the complexity of the interactions between residue management, tillage, and fertilizer management in controlling N_2O emissions from agricultural soils. This complexity has hampered efforts to develop accurate generalizations on how management changes may influence N_2O. This point is illustrated by considering recent observations of N_2O emissions from no-till systems. Several studies (Baggs et al., 2003; MacKenzie et al., 1997; Linn and Doran, 1984; Palma et al., 1997) indicate that N_2O fluxes can be greater under no-till as compared to conventional-till. Yet other studies show no significant differences in N_2O fluxes in no-till and conventional-till systems (Robertson et al., 2000; Kessavalou et al., 1998).

Implementation of no-till practices has the potential to reduce global warming potential (GWP) by increasing sequestration of soil carbon. If, however, no-till increases N_2O fluxes, then due to the increased radiative forcing of N_2O relative to CO_2 (approximately 296:1), decreases in GWP due to C sequestration may be offset by small increases in N_2O. The GWP of several agricultural systems was recently investigated by Robertson et al. (2000). In a study of corn–wheat–soybean rotations these investigators observed that the GWP of no-till management over an 8-yr period was approximately eightfold less than conventional-till (14 g CO_2 equivalents m^{-2} yr^{-1} for no-till vs. 114 g CO_2 equivalents m^{-2} yr^{-1} for conventional-till). The main differences in GWP between the two systems was the greater soil C storage under no-till (110 g CO_2 equivalents m^{-2} yr^{-1}) compared

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Abbreviations: DOY, day of year; GWP, global warming potential; IPCC, Intergovernmental Panel on Climate Change.

to the conventional-till treatment (0 g CO_2 equivalents m^{-2} yr^{-1}). The contributions of N_2O to the GWPs of these systems was nearly the same (52 g CO_2 equivalents m^{-2} yr^{-1} for conventional-till and 56 g CO_2 equivalents m^{-2} yr^{-1} for no-till).

The similar N_2O emissions observed by Robertson et al. (2000) in their no-till and conventional-till systems may be due to a temporal effect. Six et al. (2004), in a modeling assessment of N_2O fluxes from 44 conventional-till vs. no-till comparisons, found that, for their humid climate classification (dominated by sites in the U.S. Corn Belt), the changes in N_2O flux as a result of converting tilled systems to no-till changed over time. They estimated that 5 yr after the establishment of no-till, N_2O fluxes were higher than conventional-till, resulting in an increased GWP of 1114 kg CO_2 equivalents ha^{-1} yr^{-1}. After 10 yr of no-till, N_2O emissions were reduced relative to conventional-till, but still had a positive influence on GWP (330 kg CO_2 equivalents ha^{-1} yr^{-1}). However, by Year 20, N_2O fluxes from no-till were lower than in a conventional-till system resulting in a corresponding GWP reduction of GWP 1238 kg CO_2 equivalents ha^{-1} yr^{-1}.

The uncertainty associated with N_2O emissions from agricultural soils resulting from the complex interactions between residue management, tillage, and fertilizer management over varying temporal and spatial domains underscores the relevance of the recommendation by Six et al. (2004) that it is "…crucial to further investigate the long-term, as well as the immediate effects of various N-management strategies, such as precision farming, nitrification inhibitors, and type plus method of N fertilizer application, for purposes of long-term reduction of N_2O-fluxes under no-till conditions." Therefore, the objectives of this study were to (i) quantify and evaluate the N_2O emissions from corn–soybean rotations in central Iowa managed with full-width tillage, no-till, and no-till with a rye winter cover crop, (ii) characterize the temporal and spatial variations in emissions in these cropping systems, and (iii) compare our measured N_2O emissions with emissions estimated using the Intergovernmental Panel on Climate Change (IPCC) protocol.

MATERIALS AND METHODS

Study Site

A 2.11-ha field site was established in May 1995 at the Iowa State University Agronomy and Agricultural Engineering Research Center [x = 436723 y = 4652349 UTM (Universal Transverse Mercator) Zone 15 m NAD83 or 42.0200° latitude, −93.7700° longitude] located 12 km west of Ames, IA. Three predominant soils were found on the site (Andrews and Diderikson, 1981): Webster (fine-loamy, mixed, superactive, mesic Typic Endoaquolls), Clarion (fine-loamy, mixed, mesic Typic Hapludolls), and Nicollet (fine-loamy, mixed, mesic Aquic Hapludolls). A no-till system with controlled traffic was established on the entire area in 1995. In October 2001, a chisel-plow-based tillage system was established on plots comprising one-third of the area. The chisel-plow tillage system consisted of fall chisel plowing approximately 0.18 m deep with 0.076-m-wide twisted shanks and spring disking immediately before planting approximately 0.09 m deep using a tandem disk with 0.51-m-diameter disks. All machinery and foot traffic in both tillage systems were restricted to the same interrows each year and row location was maintained from year-to-year. Field plots were laid out in a randomized complete block design with 10 blocks. The field was split in half with five contiguous blocks on the west half of the field and five blocks on the east half of the field. To establish and maintain the corn–soybean rotation half of the field was planted with corn and the other half was planted with soybean each year. In the following year the crops were switched. Corn and soybean were planted in late April or early May each year with a five-row planter with a 0.76-m row spacing. At planting an ammonium polyphosphate starter fertilizer was applied in-furrow at rates of 13 and 44 kg ha^{-1} of N and P, respectively. At approximately 38 d after planting a urea ammonium nitrate fertilizer solution was applied 0.13 m from the row at a rate of 202 kg N ha^{-1}. Following both corn and soybean harvest in the fall, a rye winter cover crop was planted into selected no-till plots using a no-till grain drill with 0.19-m row spacing at 3 800 000 seeds ha^{-1}. The rye cover crop was killed in the spring 7 to 10 d before planting with glyphosate [*N*-(phosphonomethyl)glycine]. Plots were 3.8 m wide by 55.8 m long.

Soil Sampling and Analysis

Surface soil (0–30 cm) samples were collected in April 2003. Four soil cores (3.35-cm diameter) were collected from each plot and bulked. In the laboratory, samples were weighed and sieved (2 mm). Subsamples were collected for water content determination by oven drying at 105°C, and the remaining soil was air dried. Air-dried samples were ground with a roller mill for organic C and N determination by dry combustion with a Carlo-Erba NA 1500 CHN elemental analyzer (Haake Buchler Instruments, Paterson, NJ) after removal of carbonates (Nelson and Sommers, 1996). Soil pH was measured in 1:1 distilled water to soil slurries. Bulk density was computed from the soil sample weights (corrected for water content) and the known core volume. Physical and chemical properties of the soils from the three management systems are shown in Table 1. Daily precipitation totals and average air and soil temperatures were collected at a meteorology station 0.5 km west of the study area (Herzmann, 2004). Soil temperatures (5 cm) were measured at the time of gas sampling using a digital soil thermocouple temperature probe. Surface soil water content (0–6 cm) was measured using a ML2 soil moisture sensor (Delta-T, Cambridge, UK) at the times of N_2O flux measurements.

Nitrous Oxide Flux Measurements

Soil nitrous oxide emissions from the three corn–soybean cropping systems were measured from the spring of 2003 through February 2005. Four replicate plots of each treatment were sampled within both crops of the rotation in each of the two growing seasons. Nitrous oxide fluxes were measured weekly during the periods of April through October, biweekly during March and November, and monthly in December, January, and February. Two polyvinyl chloride (PVC) rings

Table 1. Soil properties of management treatments. Soil samples (0–30 cm) were collected in April 2003.

Management	pH	Bulk density	Organic C	Organic N	NO_3^-	NH_4^+
		$Mg\ m^{-3}$	$g\ kg^{-1}$ soil		$mg\ N\ kg^{-1}$ soil	
Chisel plow	6.7	1.26	15.3	1.37	7.21	1.84
No-till	6.8	1.36	15.6	1.37	4.81	1.84
No-till + rye	6.6	1.36	15.5	1.36	4.27	1.47

(30-cm diameter × 10 cm tall) were installed in each plot to a depth of approximately 6 cm. In each plot one ring was placed directly in the plant row and contained at least one plant. The other ring was placed between plant rows. These rings, which served as bases for flux chambers, were left in place during the sampling period unless they were removed for fertilization, planting, and tillage events. Flux measurements were performed by placing vented chambers (30-cm diameter × 10 cm tall) on the rings and collecting gas samples 0, 15, 30, and 45 min following chamber deployment. Chambers were constructed from PVC and covered with reflective tape. At each time-point chamber headspace gas samples (10 mL) were collected with polypropylene syringes and immediately injected into evacuated glass vials (6 mL) fit with butyl rubber stoppers. Nitrous oxide concentrations in samples were determined with a gas chromatography instrument (Model GC17A; Shimadzu, Kyoto, Japan) equipped with a ^{63}Ni electron capture detector and a stainless steel column (0.3175-cm diameter × 74.54 cm long) with Porapak Q (80–100 mesh). Samples were introduced into the gas chromatograph using an autosampler described by Arnold et al. (2001). Nitrous oxide fluxes were computed from the change in N_2O concentration with time, after accounting for diffusion effects by applying the algorithm developed by Hutchinson and Mosier (1981). Based on our precision of N_2O measurement of 5%, our estimated minimum detectable flux is estimated to be 0.00042 g N_2O-N m^{-2} d^{-1}.

Cumulative Nitrous Oxide Flux Calculations

Cumulative N_2O fluxes from each management system over the 2-yr study period were calculated. Because fluxes were measured during the daytime when soil temperatures were generally higher than the daily average soil temperatures, cumulative N_2O fluxes were calculated using temperature-corrected flux measurements. Temperature corrections were done with a Q_{10} relationship, using the 5-cm soil temperature at the time each flux was measured, along with the daily average soil temperature for that day (Parkin and Kaspar, 2003). The Q_{10} factor used in these corrections ($Q_{10} = 3.72$) was computed from diurnal N_2O fluxes measured over a 3-d period (DOY 207 to DOY 210, 2004, where DOY is day of year) using automated soil chambers similar in design to those described by Parkin and Kaspar (2003). Diurnal fluxes were measured over 1-h periods every 2 h. During the times the chambers were closed, the chamber headspace gas was recirculated through a photoacoustic detector (Innova AirTech Instruments, Ballerup, Denmark) to determine chamber headspace N_2O concentrations.

Another consideration in the calculation of cumulative N_2O fluxes is the spatial weighting of fluxes collected over fertilizer bands. The tractor traffic in the plots of this study was confined to the two outermost interrow areas of the five row plots. Because our chambers were 30 cm in diameter, and because one of the five rows in each plot received fertilizer injections on both sides of the row, due to the way the tractor had to drive down the plots, the in-row to between-row weighting was nearly equal (47.4% in-row vs. 52.6% between-row). Cumulative N_2O emissions for each ring location were calculated by linear interpolation and numerical integration between sampling times. Cumulative N_2O flux estimates for each plot were taken as the average of the cumulative fluxes of two individual rings within that plot.

Intergovernmental Panel on Climate Change Calculations

The IPCC Tier I methodology to estimate direct N_2O emissions from agricultural lands involves multiplying N inputs by an emission factor (Intergovernmental Panel on Climate Change, 1997). The first step in performing these calculations is to estimate N inputs from fertilizer:

$$F_{SN} = N_{FERT} \times (1 - Frac_{GASF}) \qquad [1]$$

where F_{SN} is the fertilizer N input to the system, N_{FERT} is the total synthetic fertilizer added, and $Frac_{GASF}$ is the fraction of total synthetic fertilizer that is emitted as $NO_x + NH_3$. The IPCC default factor of 0.1 kg NH_3–N + NO_x–N kg^{-1} of synthetic fertilizer was used for $Frac_{GASF}$. Step 2 of the IPCC methodology involves calculation of N inputs from animal wastes; however, since animal wastes were not applied in our study, Step 2 was omitted. The third step requires calculation of total N inputs in N-fixing crops:

$$F_{BN} = 2 \times Crop_{BF} \times Frac_{NCRBF} \qquad [2]$$

where F_{BN} is the N inputs from N-fixing crops, $Crop_{BF}$ is the soybean yield (kg dry biomass yr^{-1}), and $Frac_{NCRBF}$ is the fraction of N in the N-fixing crop (kg N kg^{-1} dry biomass). We used the IPCC default factor of 0.03 kg N kg^{-1} dry biomass. Finally, N inputs from crop residues are calculated:.

$$F_{CR} = 2 \times (Crop_0 \times Frac_{NCR0} + Crop_{BF} \times Frac_{NCRBF}) \times (1 - Frac_R) \times (1 - Frac_{BURN}) \qquad [3]$$

where F_{CR} is the N in crop residues returned to the soil (kg N ha^{-1}), $Crop_0$ is grain yield of non-nitrogen fixing crops (kg dry biomass ha^{-1}), $Frac_{NCR0}$ is the fraction of N in the non-N fixing crop (kg N kg^{-1} dry biomass), $Crop_{BF}$ is the yield of N fixing crops (kg dry biomass), $Frac_{NCRBF}$ is the fraction of N in the N fixing crop (kg N kg^{-1} dry biomass), $Frac_R$ is the fraction of the crop N removed from the field as crop, and $Frac_{BURN}$ is the fraction of crop residue burned. In our study residues were not burned, but grain was removed from the plots, and we used the default IPCC factor of 0.45 kg N kg^{-1} crop N for $Frac_R$. The factor of 2 in Eq. [3] is applied to account for the fact that the grain yield is roughly one-half of the total crop biomass (Intergovernmental Panel on Climate Change, 1997). In application of Eq. [3] we used the IPCC default factors of 0.015 kg N kg^{-1} dry biomass for $Frac_{NCR0}$ and 0.03 kg N kg^{-1} dry biomass for $Frac_{NCRBF}$. After all the N inputs are calculated, they are summed and multiplied by an emission factor:

$$N_2O_{DIRECT}\ (kg\ N\ yr^{-1}) = (F_{SN} + F_{CR} + F_{BN}) \times EF_1 \qquad [4]$$

where F_{SN}, F_{CR}, and F_{BN} are calculated as described in Eq. [1], [2], and [3], and EF_1 is the default emission factor for N_2O of 0.0125 kg N_2O-N kg^{-1} N input. In addition to the average EF_1 of 0.0125 kg N_2O-N kg^{-1} N input, we estimated direct N_2O emissions using the range of emissions factors (0.0025–0.0225 kg N_2O-N kg^{-1} N input) recommended (Intergovernmental Panel on Climate Change, 1997).

Statistical Analyses

Management treatment and crop effects on cumulative N_2O fluxes (plot means) were assessed using three-way ANOVA and differences assessed by Tukey's pairwise comparison. The experiment was analyzed as a split-plot design with crop × year considered as the main plot and management as the split plot. Year and crop and crop by year used reps within year by crop as the error term. Comparisons between treatments on individual sampling dates were performed using *t* tests. Analysis of row vs. interrow positional effects were determined using the Wilcoxon Signed Rank test. Statistical tests were performed with SigmaStat software (SigmaStat Version 2.03; SPSS, 2005) and SAS Version 8 (SAS Institute, 2005).

RESULTS

Nitrous oxide fluxes from each management treatment over the sampling period, along with precipitation and air temperature data, are shown in Fig. 1. Because both crops were present in each year, results of each rotation in each year are presented separately. Figure 1A shows N_2O fluxes from plots planted to corn in 2003 and soybeans in 2004. Figure 1B shows N_2O fluxes from plots planted to soybeans in 2003 and corn in 2004. Nitrous oxide fluxes exhibited pronounced temporal fluctuations, with the largest fluctuations occurring in plots planted to corn. In the corn plots the largest peaks of N_2O flux were observed in response to rainfall events after N fertilizer was applied. In 2003 corn was planted on DOY 119 and the corn was fertilized at a rate of 202 kg N ha^{-1} on DOY 155 (Fig. 1A). On DOY 161 the site experienced a 10-mm rainfall event, and on DOY 162 N_2O fluxes increased three to nine times over the

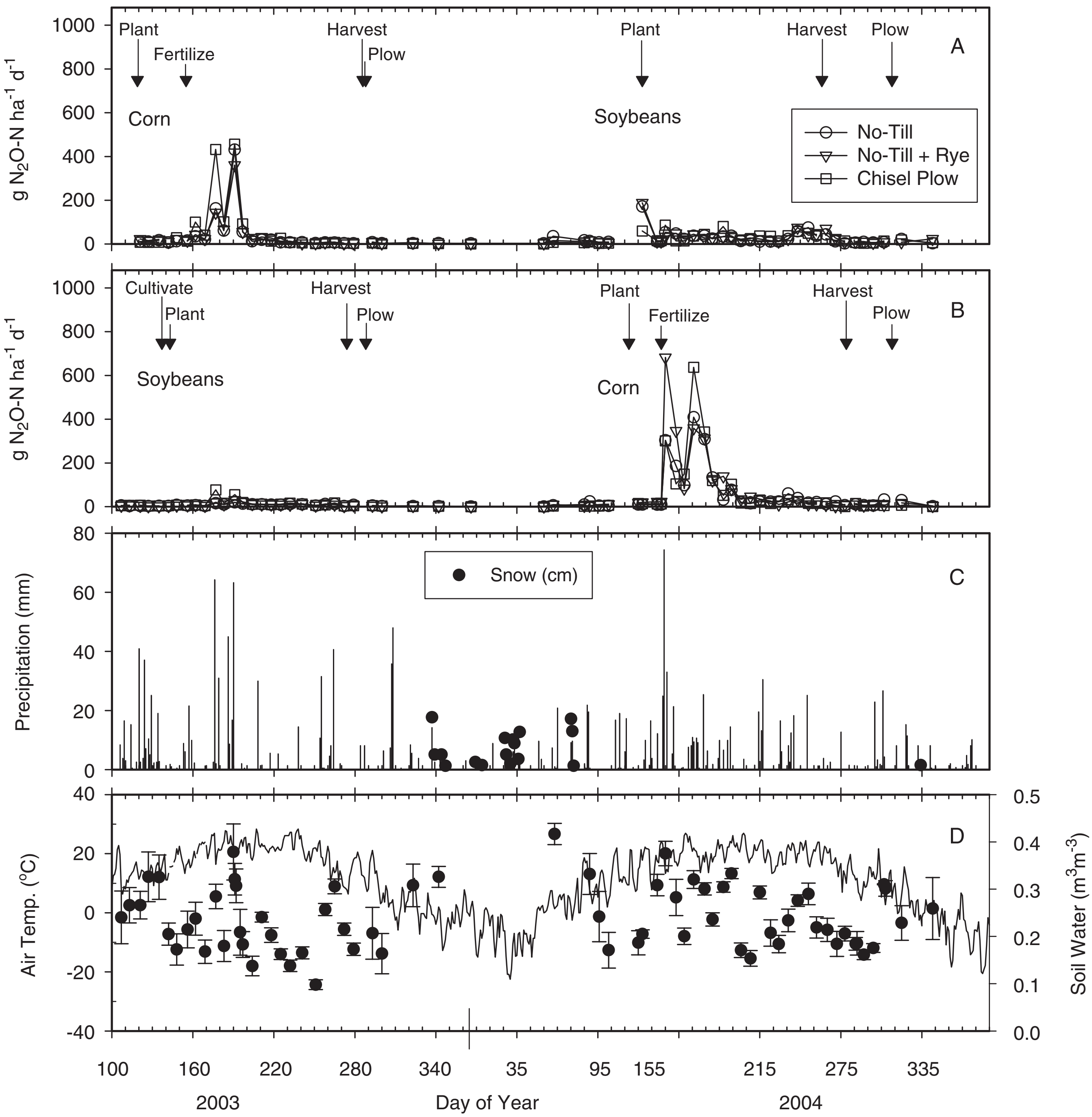

Fig. 1. Nitrous oxide emissions, daily precipitation, average daily air temperatures, and average soil water contents at sampling times throughout the study period.

previous sampling date. Larger peaks in N_2O fluxes were observed on DOY 177, following a 64-mm rain on Day 176, and on DOY 191, following rainfall events on DOY 189 (13 mm) and DOY 190 (64 mm). The N_2O emissions from corn plots in 2004 (Fig. 1B) showed a similar pattern to that observed in 2003. In 2004 the corn was fertilized on DOY 142, and following 99.2 mm of rainfall on DOY 143 and DOY 144, peaks in N_2O flux were observed on DOY 145. The next sampling on DOY 153 (Fig. 1B) followed precipitation events on Days 150–152 (>28 mm rain) and fluxes were still elevated. Between DOY 153 and 159, 2004 when the next sampling occurred, precipitation totals were <2 mm and fluxes decreased; however, fluxes increased again on DOY 166 following 28 mm of rainfall which occurred between DOY 164–166.

Nitrous oxide fluxes from the soybean plots also responded to rainfall events, but to a much lesser extent than the corn plots. In 2003 fluxes in the soybean plots ranged from 3.5 to 5.7 g N_2O-N ha^{-1} d^{-1} on DOY 166, but on DOY 177 fluxes increased and ranged from 16.4 to 77.4 g N_2O-N ha^{-1} d^{-1}. In 2004 fluxes from the soybean plots exhibited peak N_2O fluxes on DOY 145 and 153, following rainfall events.

Generally, there were few management system differences in N_2O flux. On most of the dates when fluxes were measured there were no significant management effects. Significant differences, however, were noted on some dates, but the relative differences among treatments were not consistent. In plots planted to corn in 2003 (Fig. 1A) fluxes from the chisel plow plots were significantly greater ($P < 0.05$) than either the no-till or the no-till + rye treatments on 26 June (DOY 177) and 2 July (DOY 183). These sampling dates were associated with peak N_2O fluxes. In the plots planted to corn in 2004 (Fig. 1B) the apparent differences in N_2O emissions associated with the peaks observed on 24 May (DOY 145) and 14 June (DOY 166) were not significant. Overall, in the treatments planted to corn in 2003 and soybeans in 2004 (Fig. 1A), significant treatment differences were observed on 7 of the 64 measurement dates throughout the 2-yr period. In the treatments planted to soybeans in 2003 and corn in 2004 (Fig. 1B), significant management differences were observed on four dates over the 2-yr period.

Whereas there were few management system effects on daily N_2O fluxes, within the different management systems there were positional differences in fluxes. In each plot, N_2O fluxes were measured from a pair of chambers, with one chamber of each pair positioned directly in the crop row and the other chamber positioned between rows. The average positional differences (in-row flux minus between-row flux) for each management treatment and crop are shown in Fig. 2. Over the two growing seasons, N_2O fluxes in plots planted to soybeans were significantly higher ($P < 0.05$) in the row as compared to between rows (Fig. 2A and 2B). The opposite was true for the treatments planted to corn, where between-row fluxes were significantly higher ($P <$ 0.05) than in-row fluxes during the growing seasons (Fig. 2C and 2D).

To estimate cumulative N_2O emissions from the management systems over the 2-yr study period, measured fluxes had to be corrected for diurnal temperature effects. This correction was necessary because flux measurements were only performed during the daytime (between 1000 and 1600 h) when soil temperatures were generally higher than average daily soil temperatures. A Q_{10} relationship used for this correction was derived using diurnal flux data collected during a 3-d period from automated chambers installed in a corn field in 2004 (Fig. 3). The frequencies of the diurnal fluctuations in N_2O emissions and soil temperature were similar (Fig. 3A), with maximum and minimum values of these variables corresponding at approximately the same times. The relationship between N_2O emissions and soil temperature was fit with an exponential equation to estimate a Q_{10} factor (Fig. 3B). Despite the apparent scatter in this relationship, the r^2 obtained was significant ($P < 0.05$), and the estimated Q_{10} value of 3.72 is similar to values estimated by Maag and Vinther (1999) derived from measurements of N_2O production in laboratory incubations. We also calculated cumulative N_2O emissions using fluxes that were not temperature corrected to assess the magnitude of the bias associated with sampling only during the daytime hours. We determined that the non-temperature-corrected cumulative N_2O emissions were approximately 10% higher than the temperature-corrected emission estimates (averaged over all treatments).

A summary of management effects on cumulative N_2O flux is presented in Table 2. Analysis of variance indicated that management did not significantly affect cumulative N_2O flux ($P = 0.586$). Also, there were no significant year-by-crop ($P = 0.206$), year-by-management ($P = 0.317$), or crop-by-management ($P = 0.792$) interactions. However, crop was significant in both years ($P < 0.001$). Comparison of individual crops across years revealed that N_2O flux from the soybean crop was significantly greater ($P < 0.001$) in the 2004 growing season than the soybean crop in the 2003 season. Similarly, N_2O flux from the corn crop in 2004 was significantly greater ($P < 0.001$) than emissions from the corn in 2003. While the overall crop differences are likely due to the fertilizer N additions to the corn, the significant differences between years may be attributed to precipitation patterns. Although total precipitation during the 2003 season (April 2003–March 2004) was higher than the April 2004–February 2005 precipitation (971 and 782 mm, respectively), precipitation in May of 2004, after fertilization, was nearly twice as high as in 2003 (8.2 mm and 4.8 mm, respectively). This small difference in precipitation may seem insignificant; however, 3.2 mm of water added to a soil with a total porosity of 50% and 50% water filled pore space (WFPS) would increase WFPS in the top 2 cm to 84%.

The IPCC protocol for estimation of direct N_2O fluxes from agricultural lands is based on N inputs to soil from a variety of sources, including crop residues, N fixation, animal waste, and synthetic fertilizers (Intergovernmental Panel on Climate Change, 1997). In our study, animal wastes were not included in the management systems,

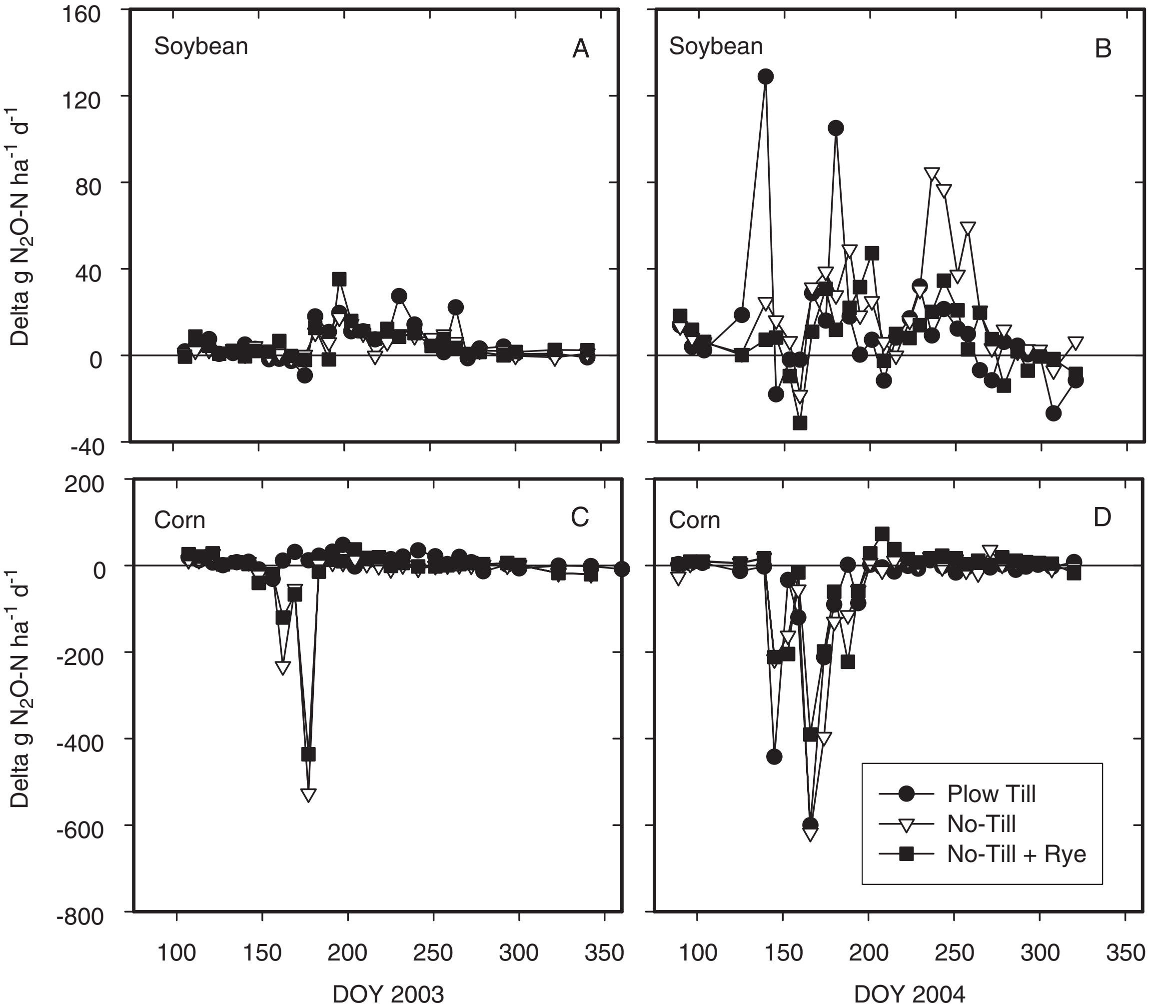

Fig. 2. Average differences between N_2O fluxes measured in the row vs. between plant rows. Positive values indicate that in-row emissions are higher than between-row emissions for the soybean plots [(A) and (B)]. Negative values indicate that in-row emissions are lower than between-row emissions in the corn plots [(C) and (D)].

thus N added was only through synthetic fertilizers, biological fixation by soybeans, and corn and soybean biomass returned to the soil (Eq. [1]–[4]). All our treatments received 215 kg N ha^{-1} applied to the corn year of a corn–soybean rotation. Grain yields and associated IPCC estimates of N returned to the soil for our study are shown in Table 3. In calculating the IPCC estimates we used our measured corn and soybean yields from 2002 and 2003, along with measured rye biomass that was produced and returned to the soil in the spring of 2003 and 2004. Over the 2-yr period of our study estimated plant biomass N returned to soil was not significantly different ($P > 0.05$) for the chisel plow and no-till; however, plant biomass-N returns in the cover crop treatment were significantly higher than in the non-cover crop treatments. The main difference for this is due to the added N from the rye residues.

The IPCC protocol for estimating direct N_2O emissions from agricultural lands is a simple multiplication of the total N returns to the land by an emission factor of 0.0125 kg N_2O-N kg^{-1} N input. We computed IPCC estimated N_2O emissions for our management treatments using this factor along with our known fertilizer N inputs and estimated plant biomass N inputs (Fig. 4). The IPCC protocol also presents a range of emission factors that bracket their default factor, from a low of 0.0025 to a high of 0.0225 kg N_2O-N kg^{-1} N input. Ninety-five percent confidence intervals for the IPCC estimates were calculated using the variability in plant biomass N inputs (as reflected by plot variability in grain yields). Confidence intervals for the measured N_2O fluxes were computed from the plot-to-plot variations in cumulative N_2O flux within the given treatments. In all management treatments, the IPCC estimates derived using the default emissions factors of 0.0025 and 0.0125 kg N_2O-N kg^{-1} N input (IPCC-1 and IPCC-2, respectively; Fig. 4) were significantly lower than measured emissions ($P < 0.05$), and in the two no-till treatments the IPCC emission estimates using the high range factor of 0.0225 kg N_2O-N kg^{-1} N input (IPCC-3; Fig. 4) were significantly lower than the measured fluxes.

It should be noted that the plant biomass factors used in these calculations to estimate plant residue N returns to the soil were the IPCC default factors for nonlegumes

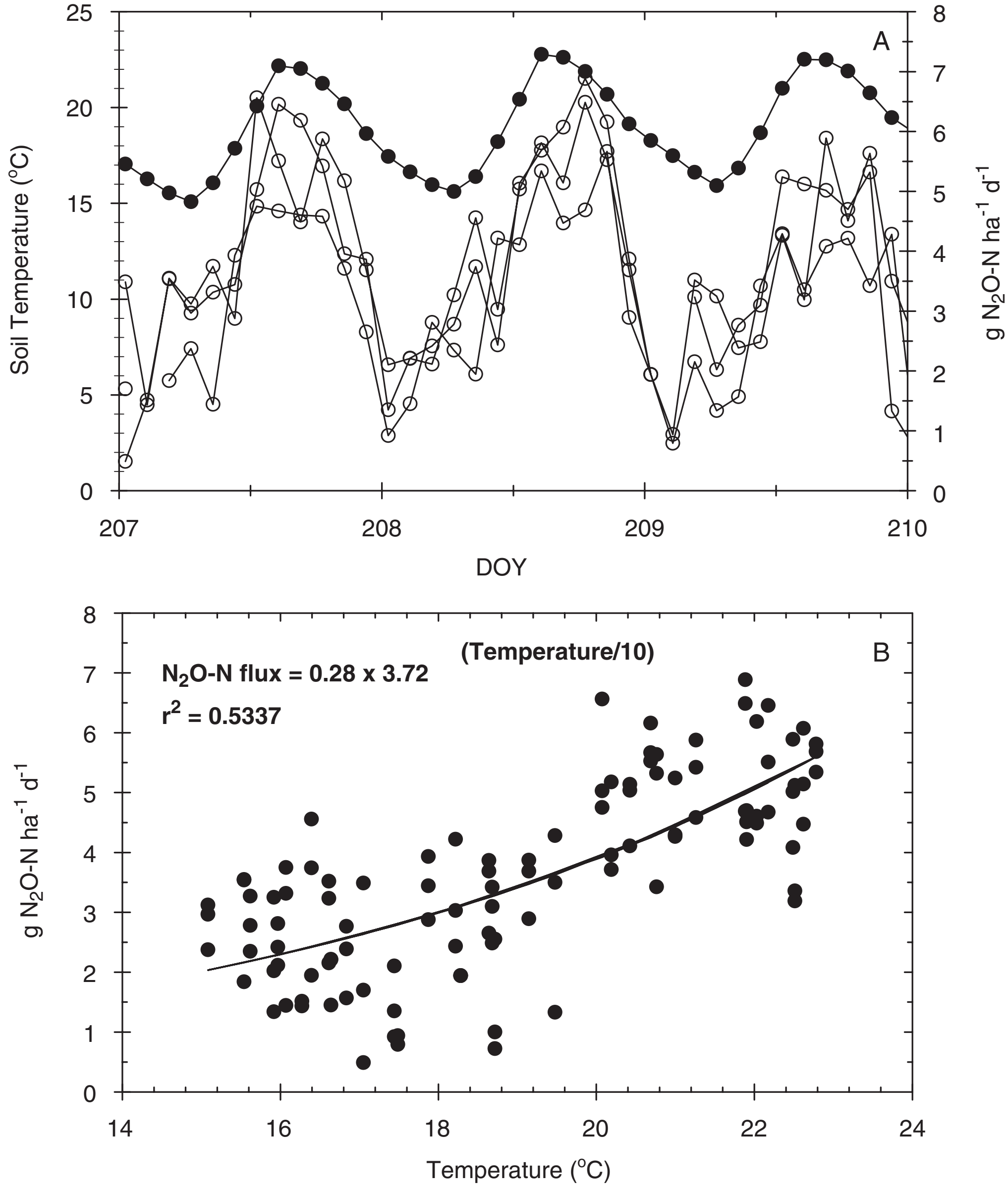

Fig. 3. (A) Diurnal variations in 5-cm soil temperature (closed circles) and N_2O emissions (open circles) from three automated chambers placed in a corn field in 2004. (B) Relationship between 5-cm soil temperature and N_2O emissions.

and legumes (0.015 kg N kg^{-1} dry biomass for $Frac_{NCR0}$ and 0.03 kg N kg^{-1} dry biomass for $Frac_{NCRBF}$). These factors are substantially higher than our measured N contents of the soybean and corn residues returned to the soil (0.0082 kg N kg^{-1} dry biomass and 0.00645 kg N kg^{-1} dry biomass, respectively). It is likely that the IPCC biomass N factors overestimate the actual amount of biomass N returned to the soil, as they appear to be based on whole plant N estimates (grain + stover), and not just N content of the stover. Our measured residue N returns to the soil based on direct sampling and analysis of the post-harvest stover N contents were 96.5, 92.9, and 158 kg N ha^{-1} for the chisel plow, no-till, and no-till + rye treatments, respectively (over the 2-yr rotation cycle). These values are substantially higher than the IPCC estimated plant biomass returns of 258, 248, and 311 kg residue N ha^{-1} for our chisel plow, no-till, and no-till + rye treatments, respectively (Table 3). Thus, using our measured plant biomass N returns, along with the IPCC emissions factors, we obtain even lower estimates of predicted N_2O-N emissions. Despite the fact that we had measured plant biomass N additions to the soil we used the IPCC estimated values because our goal was to evaluate the default assumptions inherent in the IPCC protocol.

DISCUSSION

Changing management practices in agricultural systems may offer opportunities for mitigation of global warming. In row crop agriculture, conversion of conventional-till to no-till has been shown to increase soil C, thus reducing net CO_2 emissions. However, soils under no-till management are often wetter, have decreased aeration, and have

Table 2. Cumulative N_2O flux for management treatments over 2003 and 2004 growing seasons.

Management	2003 Crop	2004 Crop	Cumulative N_2O flux† — Measurement period: 12 Apr. 2003 to 29 Mar. 2004	5 Apr. 2004 to 11 Feb. 2005
			kg N_2O-N ha^{-1}	
Chisel plow	corn	soybean	10.2 (5.80)‡	6.10 (1.04)
No-till	corn	soybean	7.87 (3.97)	6.96 (2.14)
No-till + rye	corn	soybean	7.62 (2.17)	8.21 (3.49)
Chisel plow	soybean	corn	2.71 (0.06)	11.3 (3.73)
No-till	soybean	corn	2.17 (0.30)	11.3 (2.41)
No-till + rye	soybean	corn	2.28 (0.31)	15.4 (7.33)

Three-way ANOVA results

Source of variation	DF	SS	MS	*F*	*P*
Year	1	464.4	464.4	32.2	<0.001
Crop	1	703.3	703.3	48.7	<0.001
Year × crop	1	21.7	21.7	1.50	0.232
Rep (year × crop) 12	12	124.7	10.4	0.72	0.718
Management	2	14.2	7.1	0.49	0.917
Year × management	2	31.1	15.5	1.08	0.357
Crop × management	2	6.1	3.1	0.21	0.810
Year × crop × management	2	8.0	4.0	0.28	0.759
Residual	24	346.4	14.4		
Total	47	1720			

† Cumulative fluxes were calculated from measured fluxes corrected for diurnal temperature variations using a Q_{10} = 3.72.
‡ Values in parentheses are standard deviations.

higher soil organic C levels; conditions that favor microbial denitrification (Aulakh et al., 1984; Linn and Doran, 1984; Rice and Smith, 1982). Indeed several past studies have reported larger N_2O emissions in no-till as compared to conventional-till, with some crops in some years (Baggs et al., 2003; Hao et al., 2001; MacKenzie et al., 1997; Palma et al., 1997). In contrast, other studies have reported that N_2O emissions from no-till are not different from conventional-till (Robertson et al., 2000; Kessavalou et al., 1998). Results of our study fall in this latter category. We observed no difference in total N_2O emissions in chisel plow and no-till systems over a 2-yr corn–soybean rotation. These results are consistent with a recent analysis by Six et al. (2004), who determined that the magnitude of the differences in N_2O fluxes from no-till and conventional-till systems was a function of the length of time the no-till systems were in place. These authors determined that in humid climates early after establishment of no-till (5 yr), fluxes from no-till were higher than conventional-till, but at 10 yr fluxes from the two tillage systems were nearly equal, and at 20 yr following the establishment of no-till N_2O emissions were higher than conventional-till. Our no-till treatments were established in 1995, and thus, at the time of this study, had been under no-till management for 8 to 9 yr.

Although tillage management was not a significant factor influencing total N_2O emissions in our study, the type of crop present did have a significant influence. We observed that annual N_2O fluxes from corn plots were approximately five times higher than from the soybean plots in 2003 and approximately two times higher than

Table 3. Average grain yields and estimated plant biomass N and total N applied to the management treatments over the two year cropping season. Estimated plant biomass nitrogen was determined using the Intergovernmental Panel on Climate Change (1997) protocol based on grain yield.

Management	Soybean grain	Corn grain	Rye residues†	Nitrogen applied to the soil (estimated): Plant N‡	Total N§
	kg dry grain ha^{-1}		kg ha^{-1}	kg N ha^{-1}	
Chisel plow	2822 (479)¶	9997 (277)		258 (17.2)	473
No-till	2554 (364)	9833 (683)		248 (20.2)	463
No-till + rye	2563 (369)	9728 (410)	1171 (461)	311 (13.8)	526

† Total rye residues returned to soil were computed by multiplying measured aboveground residues by a factor of 2 to account for rye root biomass.
‡ Corn and soybean biomass N returned to the field were estimated from grain yields measured in 2002 and 2003 according to Eq. [3].
§ Total N returned to the soil was computed by adding fertilizer inputs (215 kg N ha^{-1}) to the IPCC-estimated plant biomass N returns.
¶ Numbers in parentheses are standard deviations.

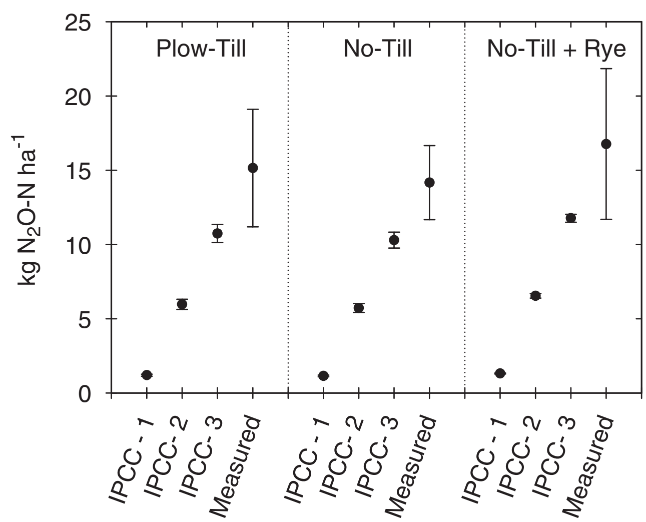

Fig. 4. Estimated and measured nitrous oxide fluxes for different management treatments. Error bars indicate 95% confidence intervals of the means. IPCC-1, IPCC-2, and IPCC-3 indicate estimates derived using Intergovernmental Panel on Climate Change emissions factors of 0.0025, 0.0125, and 0.0225, respectively.

the soybean plots in 2004. The temporal dynamics of our N_2O emission estimates indicate that the higher fluxes in the corn systems may be due to fertilizer application.

We observed peak fluxes of N_2O in response to rainfall soon after fertilization events; an observation reported by other studies (Baggs et al., 2003; Bremner et al., 1981; Cates and Keeney, 1987; Clayton et al., 1997; Goodroad et al., 1984; Jacinthe and Dick, 1997; Sehy et al., 2003). Jacinthe and Dick (1997) suggested that peak events of N_2O flux may make a substantial contribution to total N_2O emissions. The relative importance of these peak events on our annual N_2O emissions was calculated. In 2003, 49% of the cumulative N_2O flux in the plots planted to corn was due to the two peaks occurring on DOY 177 and 191. In 2004, the two peaks spanning the 29-d period from DOY 145 to 174 accounted for 45% of the annual N_2O flux from the corn plots. In contrast, the N_2O response to these rainfall events in the soybean plots was lower. In the soybean plots, the percentage of total N_2O flux corresponding to the peak events accounted for 19% of the total N_2O flux in 2003 and 8.4% of the annual N_2O flux in 2004.

Initially, we hypothesized that the rye cover crops may reduce N_2O emissions due to the rye's competition with soil microorganisms for available NO_3^-. The presence of living plants has been shown to effectively compete with soil microorganisms for available NO_3^-, thus reducing gaseous nitrogen loss (Haider et al., 1987; Smith and Tiedje, 1979). However, we observed no significant effect of the rye cover crop on annual N_2O emissions. Although soil NO_3^- and NH_4^+ concentrations in the spring were lower in the rye treatments (4.3 μg NO_3–N g^{-1} soil) compared to either the no-till or chisel plow without rye (7.2 and 4.8 μg NO_3–N g^{-1} soil, respectively), inorganic N levels were not zero. Because the rye was killed in April of each year (before fertilization), the rye would have little impact on fertilizer N applied in May, during the times of peak N_2O flux.

The corn–soybean cropping systems of our study exhibited differences with regard to spatial distribution of N_2O fluxes within the plots. In the corn years, higher N_2O fluxes were observed between rows as compared to in the row. This result in the corn plots is likely due to fertilizer placement. In our corn plots liquid urea-ammonium nitrate fertilizer was spoke-injected at 0.20-m intervals approximately 0.20 m from the row. The flux chambers positioned in the row did not include fertilizer injection points; however, the between-row chambers all included one to two fertilizer injection points. Goodroad et al. (1984), in a study of N_2O emissions from corn plots under reduced-tillage management, also observed higher emissions between corn rows compared to in-the-row, despite the fact that a surface broadcast ammonium nitrate fertilizer was used. In their study, residue was removed from the row areas during planting, whereas between the rows high amounts of crop residue remained. The coexistence of residue and fertilizer between the rows may be a critical factor controlling N_2O production in soil. This coexistence of residue and NO_3^- may also be a reason for the high N_2O fluxes observed by Baggs et al. (2003) in their no-till treatments with surface-applied ammonium nitrate fertilizer and wheat or rye residues.

In contrast to our corn treatment, our soybean plots exhibited higher N_2O fluxes in the row than between plant rows during the growing season. On average, over the 2-yr sampling period, the in-row emissions of N_2O in the soybeans were 64.3, 63.7, and 42.4% higher than between the rows for our chisel plow, no-till, and no-till + rye treatments, respectively. Explanation of the positional differences in the soybean plots cannot be attributed to fertilizer inputs as no fertilizer was applied. However, the higher in-row fluxes may have been due to the N_2O production by N_2 fixing bacteria associated with the soybeans (Stephens and Neyra, 1983; Zablotowicz and Focht, 1979). We do not attribute the higher N_2O fluxes in the soybean rows to the direct channeling soil-derived N_2O through the plant to the atmosphere as described by Chang et al. (1998). Observations of these investigators showed that this phenomenon did not occur when soils were at water contents at or below field capacity, but only when soils were saturated. None of our flux sampling events were performed when the soils were saturated. Despite the underlying mechanism controlling the higher N_2O emissions within the soybean rows, the observed positional differences may be an important methodological consideration related to chamber placement in the field.

In an effort to develop better estimates of global N_2O fluxes, the IPCC developed a protocol for estimating emissions from agricultural systems based on N inputs (Intergovernmental Panel on Climate Change, 1997). For row-crop agriculture, inputs N inputs from fertilizer, N_2 fixation by legumes, manure, and plant residues are multiplied by an average emissions factor. Application of the IPCC procedure to our results yielded lower estimates of total N_2O emissions than our measured emissions over the 2-yr cropping cycle. Obviously, two possibilities underlie this discrepancy: (i) our estimates are high, and/or (ii) the IPCC estimates are too low. While our flux estimates are high relative to other published values, they are not out of the range of fluxes published for fertilized soils in central Iowa. In a 139-d study of the effects of fertilizer on N_2O emissions, Bremner et al. (1981) observed cumulative fluxes of 15.0, 19.6, and 12.4 kg N_2O-N ha^{-1} for three Iowa soils receiving injected anhydrous ammonium fertilizer. These values are at the upper range of the annual N_2O emission estimates reported in our study for fertilized soils (Table 2). The N_2O fluxes from unfertilized soils reported by Bremner et al. (1981) ranged from 1.7 to 2.5 kg N_2O-N ha^{-1}. These values are similar to the fluxes in our soybean plots in 2003 (2.17 to 2.71 kg N_2O-N ha^{-1} yr^{-1}), but lower than our 2004 soybean plot fluxes (Table 2). It should be pointed out our estimates represent cumulative fluxes for annual time steps, while the Bremner et al. (1981) values are for a 139-d study. We suspect that a more likely reason for the discrepancy between our measured emissions and the IPCC protocol-derived estimates may be related to the emission factors used. Eichner (1990) summarized N_2O emissions from 104 field experiments and observed that N_2O emission, when expressed as a percentage of the fertilizer N applied, ranged from 0.01 to 6.84%, with the highest percentages observed in Iowa soils. This wide range suggests the possibility that the range in IPCC emission factors (0.0025 to 0.0225 kg N_2O-N kg^{-1} N input) may not be representative of all situations. It is recognized that the current emission factor approach proscribed by the IPCC methodology may be too simplistic to reflect the complex processes controlling soil N_2O flux. Indeed, the form and timing of fertilizer in relation to tillage operations and residue management, as well as position within the landscape, may have greater impacts on N_2O emissions than simply fertilizer amount (Baggs et al., 2003; Eichner, 1990; Hao et al., 2001; Sehy et al., 2003).

Nitrous oxide emissions from agricultural soils have been extensively studied, and the soil, management, and environmental factors controlling N_2O emissions have been delineated. In a review of 846 N_2O emission measurements conducted on agricultural soils, Bouwman et al. (2002) concluded that there is a wealth of information available that would enable more sophisticated prediction of N_2O flux than offered by the emission factor approach adopted by the IPCC. Possible improvements for replacing the simple emission factor approach might be a more detailed emission factor approach for which factor classes are constructed by considering soil texture, climate variables, fertilizer application rate and type, as well as residue management, as indicated in the summary presented by Bouwman et al. (2002). As an alternative to an empirical emission factor approach, mechanistic models currently under development may also be valuable (Del Grosso et al., 2002; Li, 2000).

CONCLUSIONS

Results of this study corroborate observations of some past studies on soil N_2O emissions, namely: (i) no-till management of a corn–soybean rotation did not significantly impact N_2O emissions in relation to chisel plow, (ii) N_2O emissions from soils planted to corn were higher than from soils planted to soybeans, and (iii) N fertilization is apparently the controlling factor impacting the N_2O emissions. In addition, our study indicates that positional differences occur in corn and soybean fields, with higher N_2O emissions occurring over fertilizer locations in corn, and higher N_2O fluxes occurring over growing soybean plants than in the interrow area between plants. Finally, results from our study indicate that the IPCC methodology for estimating N_2O emissions may provide underestimates, and that the source of this error may be in the relatively narrow range of recommended emissions factors.

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REFERENCES

Andrews, W.F., and R.O. Diderikson. 1981. Soil survey of Boone County, Iowa. USDA-SCS. U.S. Gov. Print. Office, Washington, DC.

Arnold, S., T.B. Parkin, J.W. Doran, and A.R. Mosier. 2001. Automated gas sampling system for laboratory analysis of CH_4 and N_2O. Commun. Soil Sci. Plant Anal. 32:2795–2807.

Aulakh, M.S., D.A. Rennie, and E.A. Paul. 1984. Gaseous nitrogen losses from soils under zero-till as compared with conventional-till management systems. J. Environ. Qual. 13:130–136.

Baggs, E.M., M. Stevenson, M. Pihlatie, A. Regar, H. Cook, and G. Cadisch. 2003. Nitrous oxide emissions following application of residues and fertiliser under zero and conventional tillage. Plant Soil 254:361–370.

Breitenbeck, G.A., A.M. Blackmer, and J.M. Bremner. 1980. Effects of different nitrogen fertilizers on emission of nitrous oxide from soil. Geophys. Res. Lett. 7:85–88.

Bremner, J.M., G.A. Breitenbeck, and A.M. Blackmer. 1981. Effect of anhydrous ammonia fertilization on emission of nitrous oxide from soils. J. Environ. Qual. 10:77–80.

Bouwman, A.F., L.J.M. Boumans, and N.H. Batjes. 2002. Emissions of N_2O and NO from fertilized fields: Summary of available measurement data. Global Biogeochem. Cycles. 16:6-1 to 6-13.

Cates, R.L., and D.R. Keeney. 1987. Nitrous oxide production throughout the year from fertilized and manured maize fields. J. Environ. Qual. 16:443–447.

Chang, C., H.H. Janzen, C.M. Cho, and E.M. Nakonechny. 1998. Nitrous oxide emissions through plants. Soil Sci. Soc. Am. J. 61:35–38.

Clayton, H., I.P. McTaggart, J. Parker, and L. Swan. 1997. Nitrous oxide emissions from fertilised grassland: A 2-year study of the effects of N fertilizer form and environmental conditions. Biol. Fertil. Soils 25:252–260.

Del Grosso, S., D. Ojima, W. Parton, A. Mosier, G. Peterson, and D. Schimel. 2002. Simulated effects of dryland cropping intensification on soil organic matter and greenhouse gas exchanges using the DAYCENT ecosystem model. Environ. Pollut. 116:S75–S83.

Eichner, M. 1990. Nitrous oxide emissions from fertilized soils: Summary of available data. J. Environ. Qual. 19:272–280.

Goodroad, L.L., D.R. Keeney, and L.A. Peterson. 1984. Nitrous oxide emissions from agricultural soils in Wisconsin. J. Environ. Qual. 13:557–561.

Haider, K., A. Mosier, and O. Heinemeyer. 1987. The effect of growing plants on denitrification at high soil nitrate concentrations. Soil Sci. Soc. Am. J. 51:97–102.

Hao, X., C. Chang, J.M. Carefoot, H.H. Janzen, and B.H. Ellert. 2001. Nitrous oxide emissions from irrigated soil as affected by fertilizer and straw management. Nutr. Cycling Agroecosyst. 60:1–8.

Herzmann, D. 2004. IEM COOP data download. Available at http://mesonet.agron.iastate.edu/request/coop/fe.phtml (verified 13 Feb. 2006). Iowa Environmental Mesonet, Iowa State Univ., Dep. of Agron., Ames.

Hutchinson, G.L., and A.R. Mosier. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci. Soc. Am. J. 45:311–316.

Intergovernmental Panel on Climate Change. 1997. Greenhouse gas inventory reference manual—Agriculture. *In* J.T. Houghton et al. (ed.) Revised 1996 IPCC guidelines for National Greenhouse Gas Inventories. IPCC, Geneva.

Intergovernmental Panel on Climate Change. 2001. Technical summary of the 3rd Assessment Report of Working Group 1. Available at http://www.grida.no/climate/ipcc_tar/wg1/010.htm (verified 13 Feb. 2006). IPCC, Geneva.

Jacinthe, P.-A., and W.A. Dick. 1997. Soil management and nitrous oxide emissions from cultivated fields in southern Ohio. Soil Tillage Res. 41:221–235.

Kessavalou, A., J.W. Doran, A.R. Mosier, and R.A. Drijber. 1998. Greenhouse gas fluxes following tillage and wetting in a wheat-fallow cropping system. J. Environ. Qual. 27:1105–1116.

Kroeze, C., A.R. Mosier, and A.F. Bouwman. 1999. Closing the global N_2O budget: A retrospective analysis 1500–1994. Global Biogeochem. Cycles 13:1–8.

Li, C.S. 2000. Modeling trace gas emissions from agricultural ecosystems. Nutr. Cycling Agroecosyst. 58:259–276.

Linn, D.M., and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci. Soc. Am. J. 48:1267–1272.

Maag, M., and F.P. Vinther. 1999. Effect of temperature and water on gaseous emissions from soils treated with animal slurry. Soil Sci. Soc. Am. J. 63:858–865.

MacKenzie, A.F., M.X. Fan, and F. Cadrin. 1997. Nitrous oxide emissions as affected by tillage, corn-soybean-alfalfa rotations and nitrogen fertilization. Can. J. Soil Sci. 77:145–152.

Nelson, D.W., and L.E. Sommers. 1996. Total carbon, organic carbon and organic matter. p. 961–1010. *In* D.L. Sparks (ed.) Methods of soil analysis. Part 3. SSSA Book Ser. 5. SSSA, Madison, WI.

Palma, R.M., M. Rimolo, M.I. Saubidet, and M.E. Conti. 1997. Influence of tillage system on denitrification in maize-cropped soils. Biol. Fertil. Soils 25:142–146.

Parkin, T.B., and T.C. Kaspar. 2003. Temperature controls on diurnal carbon dioxide flux: implications for estimating soil carbon loss. Soil Sci. Soc. Am. J. 67:1763–1772.

Rice, C.W., and M. Smith. 1982. Denitrification in no-till and plowed soils. Soil Sci. Soc. Am. J. 46:1168–1173.

Robertson, G.P., E.A. Paul, and R.R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. Science 289:1922–1925.

SAS Institute. 2005. SAS Version 8. SAS Inst., Cary, NC.

Sehy, U., R. Ruser, and J.C. Munch. 2003. Nitrous oxide fluxes from maize fields: Relationship to yield, site-specific fertilization, and soil conditions. Agric. Ecosyst. Environ. 99:97–111.

Six, J., S.M. Ogle, F.J. Breidt, R.T. Conant, A.R. Mosier, and K. Paustian. 2004. The potential to mitigate global warming with no-tillage management is only realized when practised in the long term. Glob. Change Biol. 10:155–160.

Smith, M.S., and J.M. Tiedje. 1979. The effect of roots on soil denitrification. Soil Sci. Soc. Am. J. 43:951–955.

SPSS. 2005. SigmaStat Version 2.03. SPSS, Chicago.

Stephens, B.D., and C.A. Neyra. 1983. Nitrate and nitrite reduction in relation to nitrogenase activity in soybean nodules and *Rhizobium japonicum* bacteroids. Plant Physiol. 71:731–735.

Zablotowicz, R.M., and D.D. Focht. 1979. Denitrification and anaerobic nitrate-dependent acetylene reduction in cowpea rhizobium. J. Gen. Microbiol. 111:445–448.